GENEXPERT MTB/RIF ULTRA

Dra Claudia Gutiérrez
LNR - CHLA-EP
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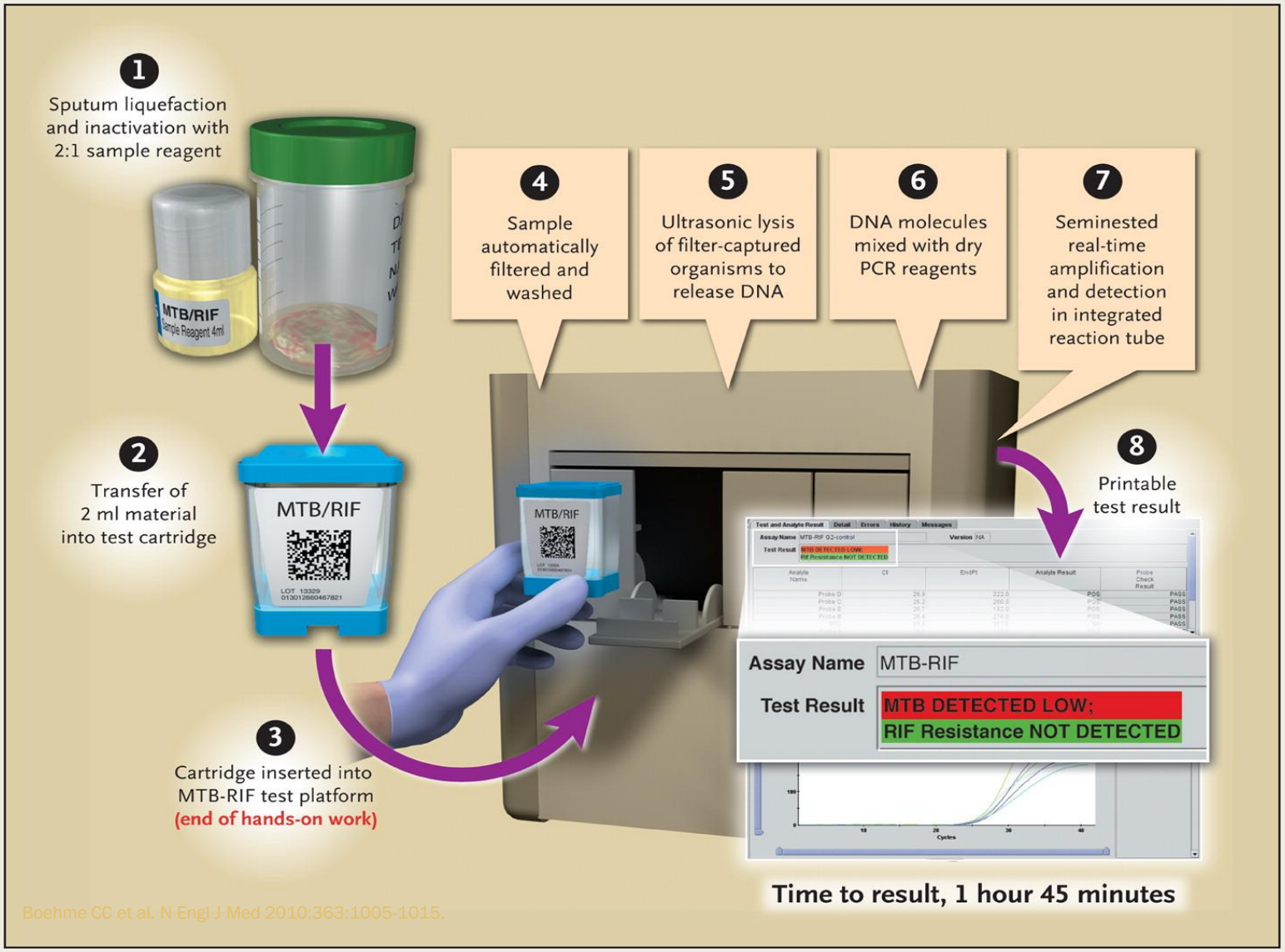
GeneXpert Xpert MTB/RIF:

- Prueba de biología molecular cerrada y automatizada, que permite detectar ácidos nucleicos del gen *rpo B* específica del complejo *Mycobacterium tuberculosis* y mutaciones asociadas con resistencia a rifampicina en menos de 2 horas.
- Es mucho más sensible que la microscopía para la detección de TB. Su sensibilidad se asemeja a la del cultivo en medio sólido.
- No detecta MNTs, a diferencia de la microcopía.
- No sirve para el seguimiento de pacientes ya que puede detectar microorganismos no viables.



Boehme CC et al. N Engl J Med 2010;363:1005-1015.

GeneXpert MTB/RIF Ultra

- Sigue el mismo procedimiento que MTB/RIF.

	Xpert MTB/RIF	Xpert Ultra
Targets	rpoB	rpoB + IS6110 and IS1081
Límite de detección	114 cfu/ml	16 cfu/ml
Detección de RR	PCR tiempo real	Análisis por curvas de fusión
Categorías de detección	High, medium, low, very low	Agrega trazas.

Que incorpora MTB Ultra?

- 2 objetivos de amplificación diferentes multicopia (IS6110 and IS1081)
- Una cámara de reacción de PCR más grande (50µl en Ultra versus 25 µl en Xpert MTB/RIF).
- Amplificación de ácidos nucleicos totalmente anidada
- Un termociclado más rápido
- Fluidos y enzimas mejorados.

Que incorpora MTB Ultra?

- Para mejorar la detección de resistencia a rifampicina incorpora un análisis mediante curvas de fusión en lugar de PCR en tiempo real.
- 4 sondas identifican las mutaciones en la region determinante de resistencia a la rifampicina del gen ropB variando la temperatura de fusión de las de la cepa wild type de referencia.

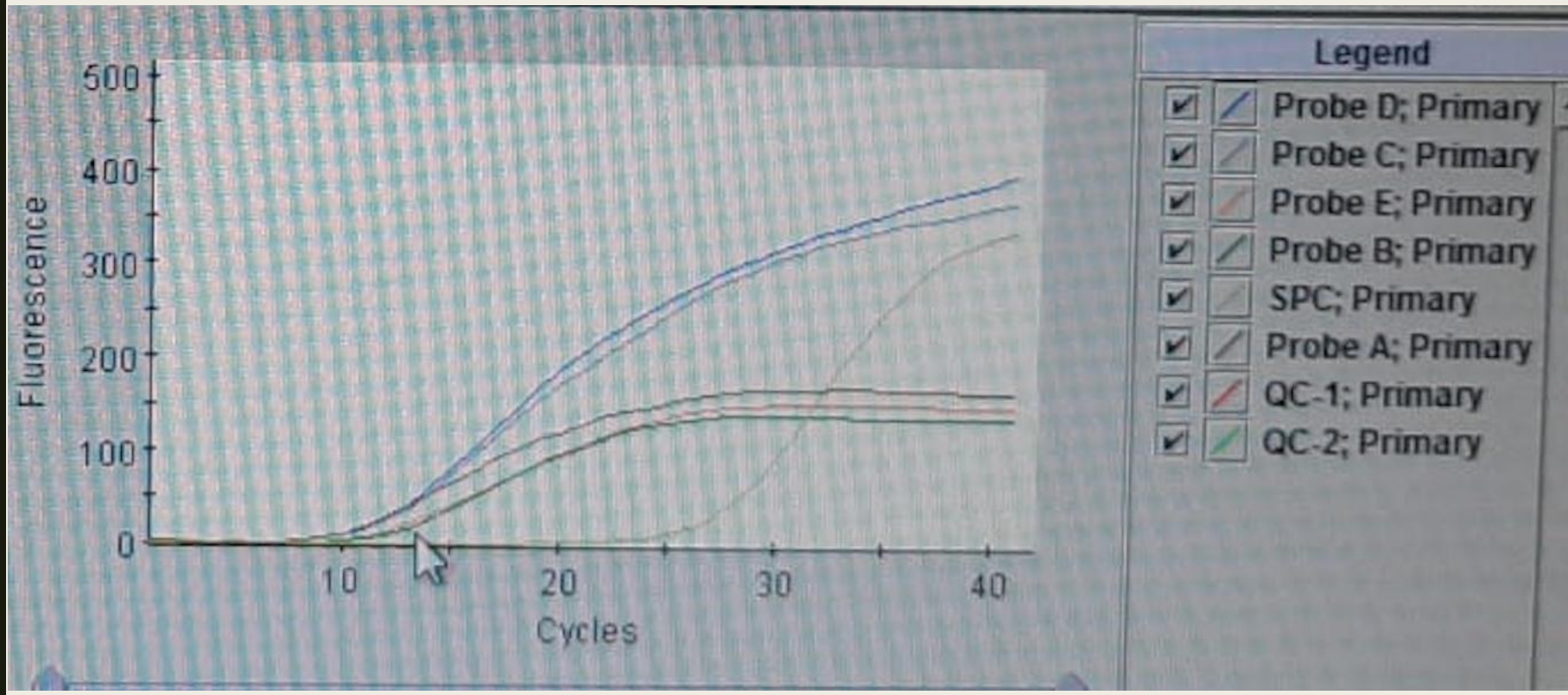
Test Result | Analyte Result | Detail | Errors | History

Assay Name Xpert MTB-RIF Assay G4 Version 5

Test Result

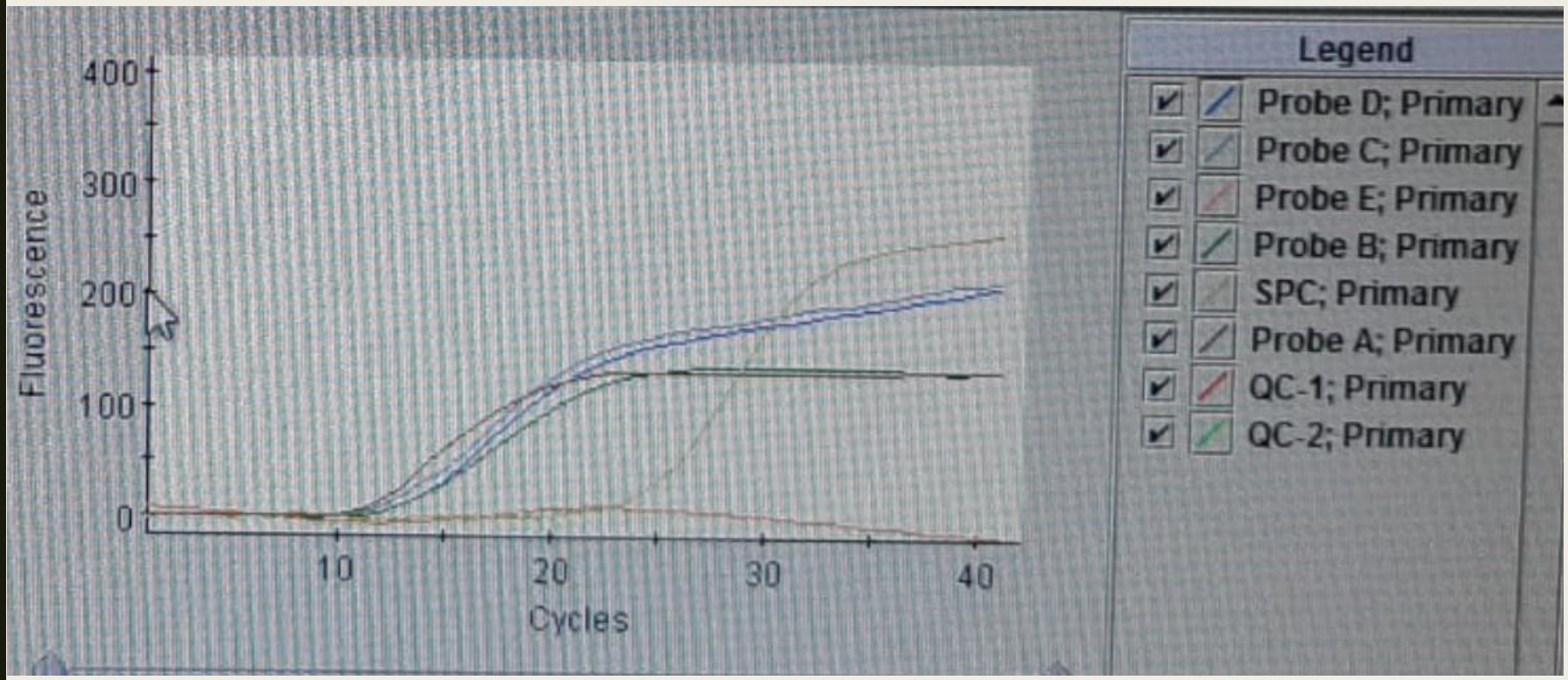
MTB DETECTED HIGH;

Rif Resistance NOT DETECTED



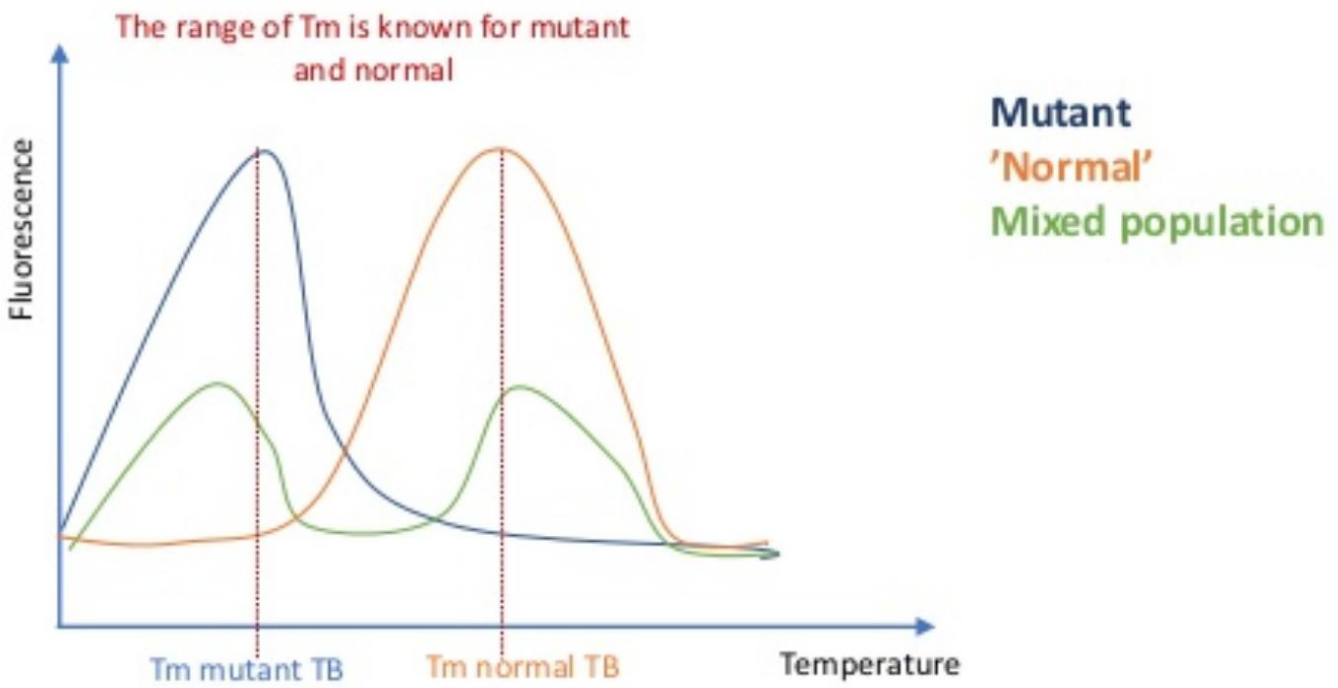
Test Result

MTB DETECTED HIGH;
Rif Resistance DETECTED



Principle of detection

Melting curve analysis: If a mutation is present, dsDNA (probe + TB DNA) dissociates sooner than if 'normal' DNA present



Cuando detecta un positivo?

- La detección *de Mycobacterium tuberculosis* (MTB) *complex* con Ultra se define como: 1 o ambas sondas de las copias multi-target son positivas con un punto de corte menor a 37 ciclos y al menos 2 sondas del *rpoB* con Cts menor a 40 ciclos.
- Un positivo trazas se detecta cuanda 1 o ambas sondas de las copias multi-target son positivas con un punto de corte menor a 37 ciclos y no mas de 1 sonda del *rpoB* tiene un Ct menor a 40 ciclos
- MTB es reportado como NO DETECTADO si ninguna de las sondas multicopia son positivas y el control interno de procesamiento es positivo con un CT menor a 35 ciclos.

Como detecta RR?

- Se considera ausente si se detecta MTB (no trazas) y las 4 sondas ropB tienen picos identificables de temperatura de fusion dentro del perfil de la cepa wild type de referencia.
- Se detecta RR si se detecta MTB (no trazas) y las 4 sondas rpoB tienen Tms identificables y al menos 1 tiene un perfil Tm mutante.
- Si MTB se detecta como trazas no puede interpretarse la RR y los resultados se reportan como RIF indeterminado.

Comparación MTB/RIF - Ultra

- Sensibilidad +5% (95%CI +2.7, +7.8)
  - *BK – Cultivo +17%, (95%CI +10, +25)*
  - *Pacientes infectados HIV+12%, (95%CI +4.9, +21).*
- Especificidad - 3,2% (95%CI -2.1, -4.7)
  - *Pacientes con antecedentes TB -5.4%, (95%CI -9.1, -3.1)*
  - *Pacientes sin antecedentes TB -2.4%, (95%CI -4.0, -1.3).*
- La reclasificación de resultados trazas como negativas en todos los casos o solo en pacientes con antecedentes deTB mitiga la pérdida de especificidad (–1.0% and -1.9% respectivamente) mientras mantiene alguna ganancia en sensiblidad (+7.6% and +15% respectivamente).
- La repetición de la prueba con una nueva muestra mitiga algo de la pérdida de especificidad, similar a reclasificar como negativo, pero mantiene lamayoría de la ganancia en sensibilidad.

Comparación MTB/RIF - Ultra

- Ambos se comportaron de manera similar en la detección de RR, siendo la especificidad de ambos cercanas al 100%.
- El analisis mediante curvas de fusión permite una mejor diferenciación de mutaciones silentes de mutaciones que confieren resistencia.

- Estudios retrospectivos demostraron que en áreas de baja transmisión la especificidad del ultra es muy elevada (99.3%, 95%CI 96-99).
- Para TBEP y TB pediátrica los estudios destacan el aumento de la sensibilidad debido sobretodo a la detección de
  - *95 vs 45 para la detección de meningitis en LCR*
  - *71 vs 47% para muestras respiratorias en niños.*

Xpert MTB/RIF Ultra for detection of *Mycobacterium tuberculosis* and rifampicin resistance: a prospective multicentre diagnostic accuracy study

Susan E Dorman, Samuel G Schumacher*, David Alland, Pamela Nabeta, Derek T Armstrong, Bonnie King, Sandra L Hall, Soumitesh Chakravorty, Daniela M Cirillo, Nestani Tukvadze, Nino Bablishvili, Wendy Stevens, Lesley Scott, Camilla Rodrigues, Mubin I Kazi, Moses Joloba, Lydia Nakiyingi, Mark P Nicol, Yonas Ghebrekristos, Irene Anyango, Wilfred Murithi, Reynaldo Dietze, Renata Lyrio Peres, Alena Skrahina, Vera Auchynka, Kamal Kishore Chopra, Mahmud Hanif, Xin Liu, Xing Yuan, Catharina C Boehme, Jerrold J Ellner, Claudia M Denkinger, on behalf of the study team†*

	Tuberculosis detection*					Detection of rifampicin resistance†	
	Sensitivity: all culture-positive (95% CI; n/N)	Sensitivity: smear-negative (95% CI; n/N)	Sensitivity: HIV-negative (95% CI; n/N)‡	Sensitivity: HIV-positive (95% CI; n/N)‡	Specificity (95% CI; n/N)	Sensitivity (95% CI; n/N)	Specificity (95% CI; n/N)
Xpert	83% (79 to 86; 383/462)	46% (37 to 55; 63/137)§	90% (84 to 94; 143/159)	77% (68 to 84; 88/155)	98% (97 to 99; 960/977)	95% (91 to 98; 167/175)	98% (96 to 99; 369/376)
Xpert Ultra	88% (85 to 91; 408/462)	63% (54 to 71; 86/137)§	91% (86 to 95; 145/159)	90% (83 to 95; 103/115)	96% (94 to 97; 934/977)	95% (91 to 98; 166/175)	98% (97 to 99; 370/376)
Difference (Xpert Ultra minus Xpert)	5·4% (3·3 to 8·0; 25/162)	17% (10 to 24; 23/137)	1·3% (–1·8 to 4·9; 2/159)	13% (6·4 to 21; 15/115)	–2·7% (–3·9 to –1·7; 36/977)	–0·6% (–3·2 to 1·6; 1/175)	0·3% (–0·7 to 1·5; 1/376)
Non-inferiority margin	Not predefined	–7%	Not predefined	Not predefined	Not predefined	–3%	–3%

Results are based on initial testing of the first sample with Xpert MTB/RIF and Xpert MTB/RIF Ultra (Xpert Ultra) assays. Uninterpretable results (contaminated cultures or non-determinate Xpert or Ultra results) were excluded from the analysis. Culture contamination averaged 4·3–7·8%, depending on sample and culture type. Non-determinate results (invalid, error, no result) are reported in the main text. Sensitivities of Xpert and Xpert Ultra for detection of smear-positive tuberculosis (n=323) were 99% (95% CI 97–100) and 99% (97–100). *Accuracy for tuberculosis detection was estimated in study participants in the case detection group. Patients with unknown HIV-infection status are excluded from analyses stratified by HIV status but included in all other analyses. †Accuracy for detection of rifampicin resistance was estimated in all study participants with available drug susceptibility test results and valid rifampicin resistance results for both Xpert and Xpert Ultra. ‡Data on HIV-infection status were not available for 188 culture-positive and 336 culture-negative study participants. Sensitivity of Xpert and Xpert Ultra in study participants with missing HIV status was 81% and 85%, respectively. Note that the estimate for pooled sensitivity of Xpert Ultra irrespective of HIV status does not fall between the estimates for HIV-infected and HIV-uninfected individuals. §Accuracy estimates are based on the reference standard as defined in the Methods section (using four cultures to define tuberculosis); using a less stringent reference standard with only one liquid and one solid culture (both from sputum sample 2), which is similar to the reference standard used in 21 of 22 studies included in the most recent Cochrane systematic review of the Xpert assay,[4] resulted in Xpert sensitivity for smear-negative tuberculosis of 73% (Cochrane review pooled estimate 67%) and Xpert Ultra sensitivity of 84% (appendix p 5).

Table 2: **Comparative accuracy for detection of tuberculosis and rifampicin resistance**

Xpert MTB/RIF Ultra for detection of *Mycobacterium tuberculosis* and rifampicin resistance: a prospective multicentre diagnostic accuracy study

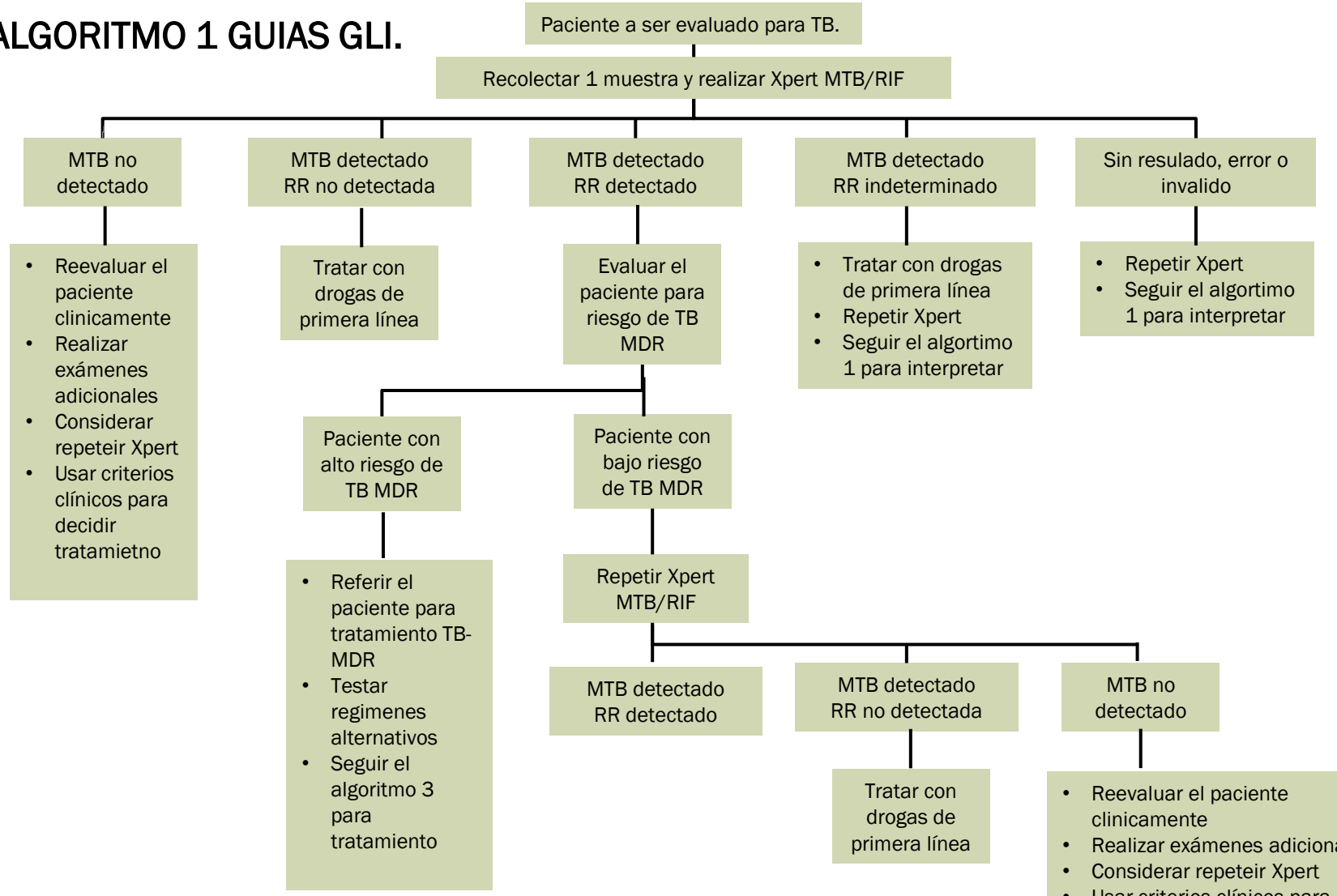
Susan E Dorman, Samuel G Schumacher*, David Alland, Pamela Nabeta, Derek T Armstrong, Bonnie King, Sandra L Hall, Soumitesh Chakravorty, Daniela M Cirillo, Nestani Tukvadze, Nino Bablishvili, Wendy Stevens, Lesley Scott, Camilla Rodrigues, Mubin I Kazi, Moses Joloba, Lydia Nakiyingi, Mark P Nicol, Yonas Ghebrekristos, Irene Anyango, Wilfred Murithi, Reynaldo Dietze, Renata Lyrio Peres, Alena Skrahina, Vera Auchynka, Kamal Kishore Chopra, Mahmud Hanif, Xin Liu, Xing Yuan, Catharina C Boehme, Jerrold J Ellner, Claudia M Denkinger, on behalf of the study team†*

	Sensitivity		Specificity		
	All culture-positive (95% CI; n/N)	Smear-negative, culture-positive (95% CI; n/N)	All culture-negative (95% CI; n/N)	No history of tuberculosis (95% CI; n/N)	Any history of tuberculosis (95% CI; n/N)
Xpert	83% (79–86; 383/462)	46% (37–55; 63/137)	98% (97–99; 960/977)	98% (97–99; 715/727)	98% (95–99; 244/249)
Xpert Ultra	88% (85–91; 408/462)	63% (54–71; 86/137)	96% (94–97; 934/977)	96% (95–98; 701/727)	93% (89–96; 232/249)
Xpert Ultra, no trace*	86% (82–89; 395/462)	54% (45–63; 74/137)	98% (96–98; 953/977)	98% (96–99; 709/727)	98% (95–99; 243/249)
Xpert Ultra, conditional trace†	88% (85–91; 406/462)	61% (53–70; 84/137)	97% (95–98; 945/977)	96% (95–98; 701/727)	98% (95–99; 243/249)
Xpert Ultra, trace-repeat‡	87% (84–90; 404/462)	61% (52–69; 83/137)	97% (95–98; 944/977)	97% (96–98; 707/727)	95% (91–97; 236/249)

Sensitivity varied little by history of tuberculosis and did not vary systematically. Data on tuberculosis history were not available for one patient. *Study participants testing tuberculosis-positive based on a trace-positive Xpert Ultra result (n=32) were reclassified as tuberculosis-negative. †Study participants testing tuberculosis-positive based on a trace-positive Xpert Ultra result were reclassified as tuberculosis-negative only if they had a history of tuberculosis (n=13). ‡Study participants testing tuberculosis-positive based on a trace-positive Xpert Ultra result had Xpert Ultra testing on a subsequent sputum specimen: if the subsequent sputum Xpert Ultra result was negative for *M tuberculosis* then the participant was reclassified as tuberculosis-negative; if the subsequent Xpert Ultra result was positive for *M tuberculosis* (any semiquantitative threshold), then the participant was not reclassified and remained tuberculosis-positive (14 out of 32 participants tested tuberculosis-negative on sample 2 and were reclassified; 14 tested tuberculosis-positive on sample 2 and were not reclassified; and four were were non-determinate by Xpert Ultra on sample 2 and were not reclassified).

Table 3: **Test sensitivity and specificity depending on tuberculosis history and different approaches to the interpretation of semiquantitative trace-positive results for *Mycobacterium tuberculosis* detection by Xpert MTB/RIF Ultra (Xpert Ultra)**

ALGORITMO 1 GUIAS GLI.

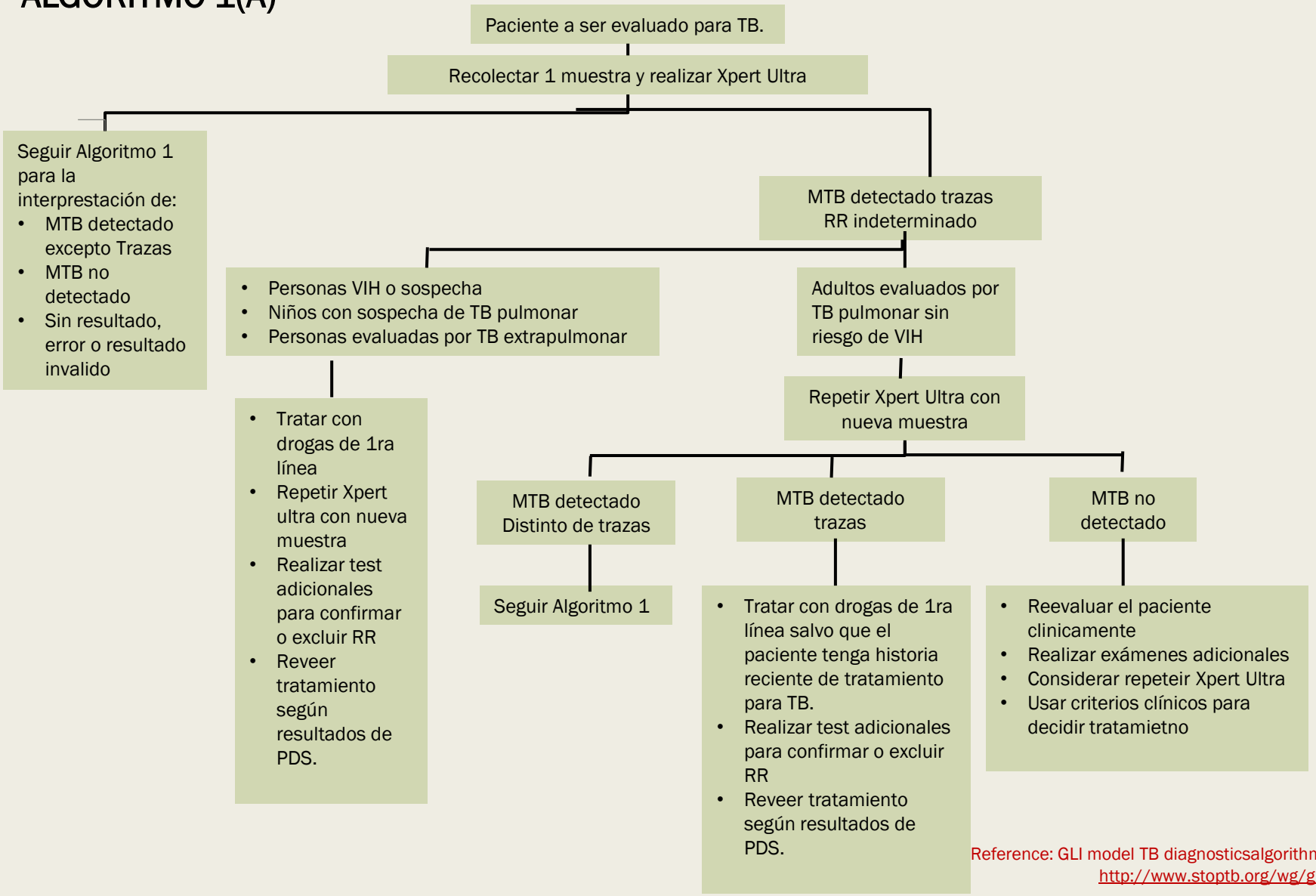


Reference: GLI model TB diagnostics algorithms
http://www.stoptb.org/wg/gli/

Cómo interpretar ”Trazas”

- Suficiente para iniciar tratamiento en personas con infeccion por VIH confirmada o sospechada, en niños y en personas con muestras extrapulmonares.
- Repetir Xpert Ultra en una segunda muestra de esputo en adultos con signos y sintomas de TB y sin riesgo de infección VIH. El segundo resultado aumentará la especificidad. El resultado del 2do test puede ser usado para guiar decisiones clínicas.
- Un 2do resultado postivo trazas se considera suficiente para realizar diagnóstico de TB pulmonar.
- Si el 2do resultado es negativo deberán realizarse mayores evaluaciones clínicas y radiológicas antes de iniciar tratamiento para TB.

ALGORITMO 1(A)



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Gracias

claudia.gutierrez@chlaep.org.uy